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ON THE CYTOLOGY OF THE ENTOMOPHTHORACEAE.

By Lincoln Ware Riddle.

With Three Plates.

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By Lincoln Ware Riddle.

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Introduction.

The Entomophthoraceae constitute a small, and, in some ways, peculiar family of Phycomycetes, including, according to Schroeter's arrangement in Engler & Prantl, seven genera. Of these seven genera, we have more or less knowledge of the nuclear conditions in four. In Conidiobolus (Brefeld, '84) we know only that the hyphae are non-septate in the early stages, but in the older stages break up into short sections (hyphal bodies) which are multi-nucleate. Basidiobolus (Eidam, '87) is perhaps the best known of the genera from a cytological point of view, chiefly on account of the work of Fairchild ('97), although the papers of Raciborski ('96), Lowenthal (:03), and Woycicki (:04) are also to be mentioned. The hyphae are here septate, dividing the fungus into cells, which are typically uni-nucleate; the conidia also possess single nuclei, derived directly without division from the parent-cells. The zygospore is formed by the fusion of the nuclei of two adjacent cells, this process being preceded, however, by the production of two beaks on the fusing cells, with a simultaneous division of the two gamete-nuclei so that a daughter-nucleus is cut off in each beak, while the other two daughter-nuclei enter the young zygospore and sooner or later fuse. Raciborski ('96[b]) and Fairchild ('97) have both described the division of the nucleus of Basidiobolus by a mitotic method.

The two remaining genera, upon which a certain amount of cytological work has been done, are Empusa and Entomophthora, all the species of which are parasitic on the larvae, pupae, or imagines of various insects, and resemble one another in their general life-history. For the general morphology of these genera reference may be made to the account given by Thaxter ('88) in his monograph of the North

American species. It was at Professor Thaxter's suggestion that the present study of the cytology of certain species of these two genera was taken up, in the hope of adding to our somewhat imperfect knowledge of this, and especially, of ascertaining the relation of the conditions involved in the formation of the resting-spores to the conditions in other Phycomycetes.

The first mention of the nucleus of Empusa is found in a paper by Maupas, published in 1879. By staining with picrocarmine, he was able to differentiate the nuclei, which, if seen by earlier observers, had been considered to be vacuoles, and to show that the hyphae of Empusa contain a large number of "petits nuclei, d'un diamètre d'environ 4 microns." The next paper was that of Vuillemin ('87), who worked with material of *Entomophthora gleospora*, which was stained in haematoxylin but was not sectioned; he describes the relatively large nuclei (10 microns in diameter) regularly spaced in the hyphae, one nucleus migrating without division into each conidium. Vuillemin made out the presence of chromatin contents in the nucleus, but found no nucleolus. In 1899 appeared a preliminary note, soon followed by the complete paper, by Cavara, describing the conditions in the hyphae and conidia of *Empusa Muscae* and *Entomophthora Delpiniana*, including also an incomplete account of the azygospores of the latter. Cavara's paper was marked by an important technical advance, inasmuch as he used microtome-sections with the most favorable stains. The following year Vuillemin (:00) published another paper on *Entomophthora gleospora*, describing the behavior of the nuclei in the formation of the azygospores, and including a theoretical discussion of certain points concerning the relationships of the genera of the Entomophthoraceae. These last two papers will be discussed in some detail in connection with the results here presented on the cytological processes in several species of Empusa and Entomophthora.

Material and Methods

In his "Monograph of the Entomophthoreae of the U. S." Thaxter ('88) combined the species usually separated into Empusa and Entomophthora in one genus under the name Empusa, considering that the simple or divided character of the conidiophores, and the presence or absence of rhizoids were features too inconstant to allow the separation of the species into two genera. Since, however, he has not been followed in this procedure by other writers, and since, further, Cavara ('99[a]) has pointed out the importance of the fact that in the species put in Empusa the conidia are typically multi-nucleate, while in those

species put in Entomophthora, they are typically uni-nucleate, it seems best to consider them as two distinct genera.

The results given in this paper are based upon a study of preparations from the following species.

1. *Empusa Grylli*, Nowakowski, on Hyphantria larvae (the "Fall Web-worm"), and on the larvae of Spilosoma.

2. *Entomophthora Americana*, Thaxter, on the imagines of Musca sp. and various other large flies.

3. Also an undescribed form closely related to *E. Americana*, found by Professor Thaxter in various localities in New England, and called in this paper *Entomophthora "x."* The form "*x*" resembles *E. Americana* in hosts and in conidia, but differs from it in the zygote, the epispore of which is smooth in *Americana*, but is furnished with bullate processes in *Entomophthora "x."* (See Figure 16.)

4. *Entomophthora echinospora*, Thaxter, on the imagines of Sapromyza (a small yellow-winged fly).

5. *Entomophthora Geometralis*, Thaxter, on the imagines of a geometrid-moth.

6. *Entomophthora rhizospora*, Thaxter, on caddis-flies.

7. *Entomophthora Fresenii*, Nowakowski, on a species of Aphis.

Most of the material was fixed in 0.75 % chrom-acetic acid, which gave satisfactory results, except for the more mature stages of the resting-spores, when it became necessary to use hot sublimate-acetic. Flemming's weaker solution was also tried, but seemed to be no improvement over the chrom-acetic mixture. It is worthy of notice, however, that material gave excellent preparations after being left in Flemming's for some weeks (cf. Chamberlain, : **01**, p. 27); the figures in Plate I illustrating mitosis were drawn from such material. Small pieces of the hosts infected with the fungus were imbedded in paraffin by the usual procedure, and sections were cut either three or six microns in thickness.

In staining, safranin-gentian-violet gave decidedly the best results. The use of orange G in connection with these stains seemed to have no added advantage. Heidenhain's iron-alum-haematoxylin was also tried, but was a decided failure, as the stain showed too great affinity for the general cell-contents. Unless otherwise stated the preparations from which the plates were drawn were fixed in chrom-acetic and stained in safranin-gentian-violet.

Entomophthora.

Nucleus.

The nucleus of *E. Americana* may be taken as typical of the genus. The resting-nucleus varies in diameter from five to ten microns, with an average of six. A successful stain gives a beautiful differentiation of the nucleus and shows its structure in considerable detail (Figure 1). The nuclear membrane is sharply defined, taking a much darker stain than the rather faint gray-blue of the cytoplasm. Within the nucleus there is a relatively small nucleolus, which stains red with the safranin-gentian-violet combination. As a rule, the nucleolus is single, but in some cases two or three may occur, when they are correspondingly smaller (Figure 13), while in other cases the nucleolus may be altogether absent (Figure 16). Around the nucleolus and occupying a large part of the nuclear cavity occurs the chromatin, in the form of densely packed granules. These usually appear evenly granular, but at times this even appearance is much less marked, and the chromatin may occur in the form of a fine meshed network (Figure 12). The chromatin in the resting-nucleus stains deeply with the gentian-violet.

The structure of this nucleus appears to be quite typical, and certainly offers no support to the statement of Cavara ('99) that "the structure of these nuclei is quite singular, and perhaps has no counterpart in those of any other plants, with the exception of the Saccharomycetes . . . the nuclei of which recall very strongly those of the Entomophthoreae." A comparison of the account of the nucleus of the yeast given by Wager ('98), which is quoted by Cavara, with the material of the present study fails to suggest the slightest similarity in the two cases. Cavara further says, "It appears that the process by which multiplication of the nuclei is effected must be ascribed to division by fragmentation or to a modification of this which is transitional to mitotic division." The present study shows that the nucleus of Entomophthora divides by a well-developed process of mitosis.

The first indication of the approach of nuclear-division is to be seen in the gradual loss of the dense, evenly granular condition of the nucleus. The chromatin becomes less densely distributed, while the granules are seen to be fewer and larger. This is followed by the gathering of more and more of these granules in the central region of the nucleus, which then shows a dense central mass of large chromatin-granules surrounded by a clear area where a few scattered granules occur, and linin-fibres are plainly visible (Figures 23, 24). Meanwhile a change in staining-reaction has taken place, the granules retaining

more and more of the safranin, so that the larger granules show a decided red. These granules then become aggregated into a definite number of spherical chromosomes (Figures 2–6). The organization of the chromosomes thus appears due to the direct aggregation of the granules, no spireme stage having been observed.

During this process of aggregation the nucleolus has taken up a position near the centre of the nucleus; but with the formation of the chromosomes the nucleolus is no longer distinguishable, since it is identical in size, shape, and staining, with the chromosomes. There is also no difference in behavior during the later stages of division. It must therefore be concluded that the so-called nucleolus of Entomophthora is in reality a chromatin-nucleolus, or karyosome. This karyosome does not contain, however, all the chromatin-content of the nucleus, as has been described by Wolfe (:**04**) for Nemalion, and by Mitzkewitsch ('**98**) for Spirogyra; on the contrary, it is equivalent to a single chromosome. The total number of chromosomes in the nucleus of *E. Americana* (including this "nucleolar chromosome") appears to be eight. This was seen clearly in many cases, and is illustrated by Figure 6 and by the lower daughter-nucleus in Figure 8. We have apparently in this species a condition where one of the chromosomes persists during the resting-stage of the nucleus as a chromatin-nucleolus. The writer has been unable to find anything comparable to this described for plants, but among animals Montgomery ('**98**) has described a similar condition in the spermatogenesis of Pentatoma.

The organization of the spindle in Entomophthora shows interesting conditions. It is always intranuclear and develops in the following manner. As the chromatin-granules draw toward the central region of the nucleus, they leave evident in the outer clear zone, numerous linin-fibres radiating in all directions from the central aggregation of chromatin (Figure 4) and running to the wall of the nucleus, which persists through all stages up to the telophase. It is highly probable that the chromatin-granules were, in the resting-period, distributed along these linin-fibres, which become evident, however, only when the granules draw toward the centre. The linin-fibres would thus be attached to the chromosomes by their proximal ends. The fibres, at first radiating in all directions, tend to separate into two groups, which gradually migrate toward the respective poles of the now somewhat drawn-out nucleus (Figure 5). These fibres form the spindle of the mitotic figure, which is thus derived wholly from the linin, and is therefore strictly intranuclear in origin and position (Figure 7). Intranuclear spindles have been described for a large number of plants

(see Davis, :04, Am. Naturalist, vol. xxxviii, p. 435, and literature there cited). Stevens (:03) has described the origin of the spindle from the linin in Synchytrium, but his material was apparently less favorable for the exact determination of the process. In Phyllactinia, Harper (:05) has shown that the spindle-fibres of the mitotic figure are identical with the linin-fibres of the resting-nucleus, but the method of spindle-formation differs from the present case, since the process is controlled by centrosomes in Phyllactinia. From the method of origin in Entomophthora, it is clear that the spindle-fibres are identical with the linin-fibres and are attached to the chromosomes permanently. In this process of spindle-formation there is no place for centrosomes, and no structures resembling centrosomes were seen in any case.

During this migration of the spindle-fibres the chromosomes are grouped in a more or less definite equatorial plate (Figure 5). The typical metaphase with the equatorial plate and the bipolar spindle, if it occurs in Entomophthora, must be of very short duration, as it is the only phase that has not been seen in examining a large number of sections showing various stages of mitosis. The nearest approach to the metaphase figure is the condition shown in Figure 5. The absence of the typical metaphase may perhaps be explained if we assume that the chromosomes split and begin to separate before the spindle becomes definitely bipolar. The next older stage of mitosis seen in the preparations studied, is one where the chromosomes have divided and the daughter-chromosomes are beginning to draw apart. The spindle, occupying the entire nuclear-cavity, elongates at this time, and the daughter-chromosomes are clearly half the size of those in the equatorial plate; this stage (Figure 7) is unmistakable and was met with repeatedly. As the daughter-chromosomes reach the poles the wall of the nucleus becomes more rounded at these points. The chromosomes remain very clear up to a late stage, and eight could often be counted.

Two methods occur in the reconstruction of the daughter-nuclei. In the first method, a vacuole appears between the two daughter-nuclei, and the spindle-fibres in this region break down, leaving only a few granules (Figure 9). The old wall of the mother-nucleus persists up to this time, connecting the two daughter-nuclei. This old wall forms the walls of the daughter-nuclei, except on the side toward the vacuole, where new walls are laid down, probably by the plasma-membrane, bordering the vacuole (cf. Lawson, :03). The portion of the old wall connecting the daughter-nuclei now disappears and the latter draw apart freely. The second method is much less common than the first, but was met with several times. The portion of the wall of the mother-nucleus and the spindle-fibres connecting the daughter-nuclei

draw apart (Figure 10), giving a very characteristic dumbbell-shaped appearance, similar to that figured by Stevens (: **01**) in Albugo. The entire wall of the daughter-nuclei in this case would be formed from the wall of the mother-nucleus. Whichever method is followed, the daughter-chromosomes remain more or less visible until after the wall is completed, when they fragment and become redistributed in the form of granules. Owing to the smaller size of the nuclei and to the density of their contents and to their strong affinity for stains at this time, it was found impossible to follow the process in detail. But it is not improbable that the process is the reverse of that followed in the organization of the chromosomes, in which case the fragmentation would be accompanied by a redistribution of the chromatin over the persistent linin-fibres.

To sum up: the process of mitosis in Entomophthora shows a chromatin-nucleolus; an organization of chromosomes by the aggregation of granules, without the intervention of a spireme-stage; the formation of a typical, intranuclear, bipolar spindle, without centrosomes, directly from the linin-fibres, which retain their connection with the chrosomomes throughout mitosis; and, in the daughter-nuclei, a redistribution of the chromosomes in a granular form over these fibres.[1]

Conidia.

The conidia of the following species have been examined and found to agree in the essential details: *E. Americana*, *E. Geometralis*, *E. echinospora*, *E. rhizospora*, *E. Fresenii*. As an illustration of the development and structure of the conidia in these species, the process may be described as seen in *E. Geometralis*.

About the time of the death of the host the hyphae break up into short multi-nucleate portions known as hyphal bodies (Thaxter, '**88**). The young hyphal body is a thin-walled structure, the cytoplasm of

[1] Since the completion of this work, two papers have appeared by E. W. Olive, under the general title, "Cytological Studies on the Entomophthoreae" (Botanical Gazette, **41**, 192–205 and 229-258, with pls. 14–16). The first paper presents a discussion of certain points in the life-history of various species of Empusa, in particular a new species, *E. Sciarae*, with a general confirmation of the results of previous workers in regard to the details of conidium formation. The second paper discusses cell- and nuclear-division. Olive finds that cell-division takes place through the entrance of a ring-shaped cleavage-furrow. The account of nuclear-division is in striking contrast to the conditions seen in the course of the present investigations, so much so that a close comparison is impossible. Olive describes what he calls a "primitive mitosis" resembling that of the lower Protozoa, a process controlled by "intranuclear centrosomes" and without definitely organized chromosomes.

which shows a more or less definite structure, which Cavara ('99) has considered to be reticulate, but which, in the preparations studied, is distinctly alveolar. This difference in appearance may be due to different nutritive conditions, or more probably to difference in fixation. The nuclei are relatively few, and show, as a rule, regular spacing. Each hyphal body sends up a long hypha to the surface of the body of the host. Into this hypha pass a varying number of nuclei. In the conidiophore, as this hypha may be called, the nuclei attain their greatest size (Figure 19), owing, in all probability, to the active metabolism necessary in connection with the formation of the conidia. The nuclei in the conidiophore, as in the hyphal body, show a strikingly regular spacing, and in the older portions a septate condition is occasionally to be seen, these septa cutting off mostly bi-nucleate cells (Figure 17). At the top of each branch of the conidiophore the cytoplasm becomes much denser, showing an upward pressure and condensation at that point. Under this pressure a small protuberance soon appears, forming the young conidium, into which passes the dense cytoplasm (compare Empusa, Figure 24). A single nucleus enters the young conidium, becoming smaller and denser during its passage. The conidium is then cut off. There is never any nuclear-division during this process, and the single nucleus of the conidium comes directly from the conidiophore. The mature conidium (Figure 18) is a comparatively thin-walled structure, densely filled with very fine-meshed cytoplasm and with a single much condensed nucleus.

Zygospores.

The formation of the zygospores has been studied by the writer in *E. Americana*, and the results there obtained have been confirmed by a further study of *E. echinospora* and *E. Geometralis*. There has been hitherto no account of the cytological processes involved in the formation of sexual resting-spores in any of the genera of the Entomophthoraceae, with the exception of Basidiobolus, in which the conditions have been generally recognized as peculiar and difficult to bring into relation with the processes known in the other Phycomycetes.

Entomophthora Americana shows two methods of zygospore formation. In the first case two hyphal bodies fuse at a point near their tips (Figure 12); the young zygospore then buds out at this point of fusion, in a manner similar to that seen in Piptocephalis, among the Mucorales. In the second case, the fusion of the two hyphal bodies is distinctly lateral (Figure 11), forming what may be compared to an H with a very short bar. The young zygospore in this case buds out from one of the gametes, at a point usually far removed from the point

of fusion. This condition is comparable to that seen in the case of *Syncephalis nodosa* (compare description by Thaxter, '97).

In both of these methods, however, the essential cytological conditions are the same. The two hyphal bodies fuse, the wall breaking down between them, and the cytoplasm becoming completely continuous, so that no line of fusion is discernible. The wall near the point of fusion, in the first case, or the wall of one of the hyphal bodies at some other point, in the second case, then swells, and this swelling, under the pressure of inflowing protoplasm, continues until a definite ampulla is formed. The wall of this ampulla is only slightly thicker than that of the hyphal body from which it is derived, and forms, as will presently be shown, the epispore of the zygote. Into this ampulla pass all the nuclei of both the fusing hyphal bodies (see Figures 11–13). It appears also that most, if not all, of the cytoplasm also passes into the young zygospore. Figure 11 shows the much vacuolated cytoplasm of the hyphal bodies; and Figures 13 and 14 show young zygospores attached to empty hyphal bodies.

The fusing hyphal bodies are accordingly multi-nucleate structures, the entire nuclear and cytoplasmic contents of which pass into the young zygospore. The hyphal bodies are therefore strictly gametes, and since they are multi-nucleate, are certainly to be considered coenogametes. Since the coenogamete is a type of sexual organ which has been shown by Davis (:00), Stevens (:01), and others to be characteristic of the Phycomycetes, the establishment of its occurrence in the Entomophthoraceae is a matter of great importance.

After the entire contents of the two gametes have passed into the young zygospore, the latter is cut off by a cross-wall. The exact method of the formation of this wall has not been determined, but stages, such as are shown in Figure 14, prove that the zygospore is not abjuncted by a process of constriction, as is the case with the conidiospore. At this time the zygote is a spherical body surrounded by a single thin wall. The cytoplasm is alveolar, and the relatively few (probably not more than eighteen) large nuclei are irregularly placed, with no apparent definiteness of arrangement. No fusion of these nuclei has taken place up to this period of formation.

The next process of interest in the development of the zygote is the formation of the thick endospore wall. As has been mentioned above, the epispore is derived directly from the wall of the hyphal body. The endospore, however, does not appear until after the zygote is cut off by a cross-wall and the old walls of the hyphal bodies have disappeared. The first sign of the formation of the endospore is the marked increase of vacuolation in a zone just inside the epispore (Figure 15). On the

inner side, this clearer zone is marked off by a densely granular region. The conditions at this period suggest strongly the periplasm found in so many Phycomycetes. This appearance, however, may be accidental and without special significance. From a study of such stages as are illustrated by Figure 15, it is evident that the granular cytoplasm is being gradually transformed into the clear material of the mature endospore (Figure 16). The endospore in Entomophthora thus appears to be formed, not as a secretion of the outer plasma-membrane, but by a direct transformation of the outer zone of cytoplasm, this transformation evidently involving more than the plasma-membrane itself, and possibly representing a primitive type of periplasm.

After this formation of the endospore, the zygote enters into a resting period which lasts until the following summer. Sections cut from material fixed three months after formation show that no fusion of the nuclei has taken place up to that time (Figure 16). Whether or not fusion of the nuclei in pairs takes place at the time of germination, as we might expect, it has been impossible to determine, owing to the difficulty of germinating the zygospores under artificial conditions. That the process of zygospore formation thus given for *E. Americana* is confirmed in full by *E. echinospora* may be seen by comparing Figures 11 to 16 with Figures 20 to 22.

It will be seen from the foregoing description that the processes connected with the formation of the sexual resting-spores in Entomophthora are closely comparable with the corresponding processes in the Mucorales, in so far as they have been made known by the work of Gruber (:01) on Sporodinia. Entomophthora has, in common with Sporodinia, coenogametes (multi-nucleate sexual cells) which are of a simple type, inasmuch as there is no differentiation of the contents and all of the nuclei function as gamete-nuclei.

Empusa.

Material of *E. Grylli* on two different hosts furnished the preparations upon which was based the study of this genus. Under *E. Grylli*, Now., Thaxter ('88) included as a synonym *E. Aulicae*, Reichhardt (in Bail, '69) first described on *Euprepia aulica*, but also occurring on other hosts, and especially in the United States, on the larvae of Spilosoma. Several authors (Cohn, '75; Schröter, '86; Lindau, '97) have kept this as a distinct species. The writer, in the course of the present study, has seen no such differences as should be considered of specific rank, and therefore prefers to follow Thaxter and consider both of the above forms as true *Empusa Grylli*, Nowakowski. The results here given are based

on material on the Spilosoma larvae; but these have been confirmed in every particular by material from the larvae of Hyphantria.

Nucleus.

The nuclei of Empusa are smaller (average diameter 4 microns), more numerous in each hyphal body than in Entomophthora, and show no regularity of spacing (Figure 23). The smaller size of the nucleus makes it a less favorable object for study; so far as has been observed, however, the structure of the nucleus of Empusa agrees perfectly with that of Entomophthora. Although caterpillars killed by *E. Grylli* were fixed at various hours of the day and night, in various conditions of moisture, and at longer and shorter periods after death, no nuclear-divisions were seen in any case. It is therefore impossible to state whether or not the details of this process also agree with those in Entomophthora, as we should expect from the close relationship of the two genera.

Conidia.

The general process of the formation of the conidia in Empusa (Figures 24 and 25) is similar to that already described for Entomophthora. There are a few differences, however, the most important of which is the fact that the conidia of Empusa are multi-nucleate structures, and the conidiophores are unbranched.

Some time before the death of the host the hyphae break up into hyphal bodies in the usual manner; and, at the time of death, each hyphal body sends up a conidiophore. In this case, however, the conidiophores and consequently the conidia are much larger than in Entomophthora, conditions correlated with the absence of branching in the conidiophore, and with the large amount of nuclear substance in the multi-nucleate conidium. The nuclei in the conidiophore are very numerous, and show no evidence of regular spacing (Figure 24). There is the usual accumulation of dense cytoplasm in the tip of the conidiophore as it swells to form the conidium. It will be recalled that in Entomophthora a single nucleus passes into the young conidium at this time. In the case of Empusa, a large number of nuclei (about twelve to fifteen) pass in and the conidium is cut off. No nuclear-division takes place during this process of conidium-formation.

After the discharge of the conidium, the nuclei left in the conidiophore degenerate, certain stages of this degeneration presenting a misleading resemblance to the prophase of nuclear-division. The nuclei can, however, be followed through all the stages of the process, ending as vacuoles containing a few oil-like globules.

The centre of the mature conidium is pretty constantly occupied by a single, large, well-defined vacuole around which the numerous, much condensed nuclei are most often clustered (Figure 25).

The conidia of *Empusa Grylli* are, then, multi-nucleate structures and corroborate the statement of Cavara ('99[a]), based on his examination of *E. Muscae*, that a multinucleate conidium is characteristic of the genus Empusa, while, as the same author shows in *Entomophthora Delpiniana*, and as has been shown above for a number of other species of the genus, Entomophthora has constantly a uni-nucleate conidium. The conidia of Empusa are also to be compared to those of Albugo and other genera of the Peronosporales, where, in all forms which have been examined (Ruhland, :03), the conidia are multi-nucleate. But the absence of a nuclear-division preceding conidium-formation in Empusa is in marked contrast to that of Albugo, a difference to be explained by the fact that, while in Albugo a series of conidia are formed, in Empusa but a single conidium is formed from each conidiophore.

Azygospores.

Just as the hyphal bodies and conidia of Empusa are multi-nucleate structures, so also we find multi-nucleate conditions in the azygospores. This multi-nucleate condition appears from the first formation stages, and these nuclei undergo neither divisions nor fusions. As these observations are not in accord with those of previous workers on the azygospores of Entomophthora, namely Cavara ('99) on *E. Delpiniana* and of Vuillemin (:00) on *E. gleospora*, it will be well to examine their results before describing the development of the azygospores in Empusa.

In *Entomophthora Delpiniana*, a species in which the hyphae do not break up into hyphal bodies, Cavara observed two sorts of structures which he called azygospores. First, a thick-walled, intercalary organ with granulated cytoplasm which shows clear reticulations, the joints of the meshes being occupied by "chromophilous granules"; this organ contains no definite morphological nucleus, but Cavara regards the "chromophilous granules" as a diffused nucleus. Such an intercalary organ appears not to have been seen by any other workers. Certainly in the present study no structures have been seen resembling Cavara's description, so that an interpretation of these curious conditions is here out of the question. Secondly, and more commonly, Cavara finds an organ of acrogenous formation; a hypha swells at the end into an ampulla; into this ampulla passes a single nucleus; a cross-wall is then formed cutting off the spore, which is, therefore, at this time, uni-nucleate; this single nucleus divides to

four, six, eight, and perhaps more, since Cavara was unable to follow the process in its further development.

Vuillemin (: **00**) states that the early development of the azygospores of *Entomophthora gleospora* is the same as in the acrogenous azygospores described for *E. Delpiniana*. There is an ampulla into which passes a single nucleus, which after the formation of a cross-wall, divides to four, six, eight, and then to sixteen. It appears, however, that this number is by no means constant, as Vuillemin records finding twelve, fourteen, fifteen, seventeen, or eighteen, which he supposes to be due to the failure of some of the nuclei to divide, or to a greater number of divisions than usual. After the nuclei have reached this number, there occurs successive fusion in pairs till the number is reduced to two; the azygospore rests in this condition for some time, but these two also ultimately fuse so that the mature azygospore is once more uni-nucleate.

Vuillemin gives no figures for any of these numerous nuclear-divisions and nuclear-fusions. His evidence seems to be based upon the different numbers of nuclei in different cases and in their smaller size when the number is greater. The number of the nuclei, however, according to the results to be described presently, is closely dependent upon the number in the hyphal body from which the azygospore is formed. And the difference in size, apart from individual variations, within restricted limits, which seem to be quite common (cf. nuclei in Figure 26), might easily be explained by the maintenance of the equilibrium of nucleo-cytoplasmic relations, the importance of which has been shown by the recent work of Gerassimow (: **01**, : **02**, : **04**), Hertwig (: **03**), and others. As Vuillemin seems to consider the conditions described to be typical for the family, it will be well to compare the account given above with results obtained from a study of the azygospores of *Empusa Grylli*.

After the hyphae of the fungus have broken up into hyphal bodies, the formation of azygospores takes place. At some point on the hyphal body, not necessarily at one end, a small protuberance appears, into which there is a flow of protoplasm from the hyphal body; the protoplasm stains more densely in this region and shows pressure. On account of this pressure the protuberance continues to expand into a rounded ampulla, the wall of which is at first only slightly thicker than that of the hyphal body (Figure 26). Into this ampulla gradually passes all of the cytoplasmic contents of the hyphal body, together with all the nuclei, which the hyphal body originally contained. Figure 26 shows a stage where this process is under way; and Figure 27, a stage where it is completed. It will be seen here that the young

azygospore is a multi-nucleate structure; and the attached empty hyphal body together with the absence of a cross-wall proves that this multi-nucleate condition is brought about, not by the successive divisions of a single primordial nucleus, but rather by the inclusion in the azygospore of the entire number of nuclei which were present in the hyphal body. As this number is, within certain limits, somewhat variable, the number of nuclei in the azygospore is correspondingly variable.

The next stage in the formation of the azygospore is marked by the formation of a cross-wall, cutting off the recently formed resting-spore; this being immediately followed by the dissolution of the walls of the empty hyphal body leaving the rounded azygospore, which at first often shows the position of the cross-wall (Figure 28). The cytoplasm is reticulated in structure, the meshes being comparatively small, and clearly granular; the wall is still only slightly thicker than that of the hyphal body, but the thickening process begins immediately.

An additional piece of evidence against the occurrence of nuclear-division within the azygospore is the fact that all of the stages described above, from the first sign of a small protuberance up to the fully formed spore, may be found on a single slide. Preparations of material in which the spores had been formed from one to several days were also studied. Yet in no case was there the slightest evidence of nuclear-divisions. Further, the number shows no striking variation, although it is often difficult to follow a spore through all the successive sections in which it should appear; and, likewise, there is no marked diminution in the size of the nuclei, such as would take place during nuclear-division (as is shown by comparing the mother-nucleus with the daughter-nuclei in Figures 1 to 10).

Finally, preparations were made from azygospores, three months old at the time of fixation. A median section of one of these is shown in Figure 29. The thinness of the wall in this case is anomalous and difficult to explain, since the normal procedure is for the wall to thicken immediately after the formation of the cross-wall, the mature azygospore having a thick wall with epispore and endospore differentiated similar to that described for Entomophthora. The appearance of this spore, fixed three months after formation (Figure 29), will be seen to be clearly similar to that of the newly-formed spore (Figure 28). The spore has increased in size somewhat, but with a corresponding increase in the vacuolation of the cytoplasm, these vacuoles being filled, in the living material, with oil-globules. The nuclear conditions are essentially identical with those in the young azygospore, and show clearly that no nuclear-divisions, and, likewise, no nuclear-fusions, have taken place up to this time.

When it is recalled that in *Entomophthora gleospora*, which, as has been mentioned, Vuillemin considers typical, the young azygospore, after the formation of the cross-wall, is uni-nucleate, and that the nuclear-divisions and fusions described take place immediately, it will be seen that here, in *Empusa Grylli*, we have a very different type of resting-spore formation — a resting-spore multi-nucleate from the beginning and showing neither nuclear-divisions nor nuclear-fusions.

The evidence seems to indicate that the azygospore of Empusa is more in the nature of a chlamydospore; and the occasional substitution of an encysted hyphal body, such as is shown in Figure 30, as a means of tiding over the winter period, is in favor of this view.

Conclusions.

In conclusion it will be well to consider what light the observations here set forth may throw on the interrelationship of the genera of the Entomophthoraceae, and the relationship of this family to other Phycomycetes. In comparing the nuclear conditions in the three genera, which have been studied from the cytological point of view, namely Basidiobolus, Entomophthora, and Empusa, two possible lines of development are offered for consideration. First, it is possible that Basidiobolus, with its uni-nucleate cells, may be a primitive type, from which the development may have proceeded through the conditions seen in the hyphal bodies of Entomophthora, with their several nuclei regularly spaced, to the conditions in Empusa, where the hyphal bodies have a large number of irregularly distributed nuclei. Or, secondly, it is possible that Empusa is the primitive type of a series showing progressive nuclear reduction, which reaches its highest expression in Basidiobolus.

The first of these two views is supported by Vuillemin in his paper of 1900. He says: "Au point de vue cytologique, l'*E. gleospora* (ainsi que l'*E. Delpiniana*) forme un trait d'union entre les Basidiobolus et les Empusa; il nous montre par quelle gradation la structure cellulaire s'est dans la série des Entomophthorées pour passer à la structure multi-nuclée. Cette dernière apparaît alors comme un dérivé phylogénétique de la première : c'est ce qui m'a depuis longtemps suggéré l'idée de la nommer une structure apocytique. Ceci posé, nous interpreterons les vues de Raciborski en disant que la famille des Entomophthorées chevauche sur les Archimycètes et les Phycomycètes, qu'elle prend ses racines dans le premier groupe au niveau des Basidiobolus, franchit la frontière au niveau des *Entomophthora gleospora* et *Delpiniana* pour s'epanouir en pleins Phyco-

mycètes au niveau des Empusa. Les détails publiés par Cavara sur la structure de l'*Empusa Muscae*, détails que j'ai pleinement vérifiés, montrent clairement ce dernier stade de l'évolution apocytique des appareils végétatif et conidien des Entomophthorées." Vuillemin thus makes Basidiobolus the primitive form for the family. But such a primitive form should show some points of relationship to the forms from which it has itself been derived. These points further should be shown in connection with the sexual processes, the period of the life-history, which is generally agreed to be of the greatest phylogenetic importance. In connection with his views Vuillemin offers no special form to which Basidiobolus might be directly related.

Such a line of evolution, as Vuillemin suggests, has been discussed by Davis (:**30**) in connection with his consideration of the origin and evolution of the coenogamete. Davis says: "It is conceivable that a uni-nucleate sexual element might become multi-nucleate perhaps through such an increase in the protoplasmic content that more than one nucleus would be required to control satisfactorily its activities. This possibility has absolutely no evidence in its support. There is no series of forms whose sexual cells pass from a uni-nucleate condition to a multi-nucleate. There are no indications that such an evolutionary process has ever taken place among plants."

So much for negative evidence against such a process as Vuillemin supposes to have occurred. Let us now see what positive evidence there is for the second of the two views stated above. The most interesting piece of such evidence, because the most complete, is that of the Albugo series established by Stevens (:**01**) (and also quoted by Davis (l. c.)). Here the progressive increase in the development of the physiologically important coenocentrum, and the corresponding decrease in the now functionless receptive papilla indicate clearly the line of evolutionary advance. While along with this, we have a progressive reduction in the nuclei, starting with *Albugo Bliti* with many functional gamete-nuclei, through *A. Tragopogonis* with many potential but only a few functional, and ending with *A. candida*, with its single functional gamete-nucleus. This view may now be applied to the conditions in the three genera of the Entomophthoraceae.

In Empusa, the originally non-septate hyphae early break up into short segments, the hyphal bodies, which contain large numbers of small nuclei distributed irregularly through the plasm. The conidia are multi-nucleate. A large number of nuclei enter the apogamous resting-spore. In Entomophthora the hyphae usually break up into hyphal bodies, but as has been shown above in the case of *E. Geometralis* and as Cavara has shown in *E. Delpiniana*, the formation of hyphal

bodies may not take place, in which case the hyphae often become septate. These septa cut off cells containing relatively few, regularly spaced nuclei which from their larger size have evidently an increase of efficiency. The tendency toward nuclear reduction has extended to the conidium resulting in a uni-nucleate condition. The resting-spores are formed by the fusion of coenogametes, with relatively few nuclei, — these coenogametes being of a simple type, where all of the nuclear material enters the zygote. In Basidiobolus the hyphae are regularly septate and do not normally form hyphal bodies. It has been shown, however, by Raciborski ('96[a]) that under certain culture conditions such a formation of hyphal bodies may be induced. The tendency to nuclear reduction has here reached its highest expression, resulting in uni-nucleate cells. Accordingly the zygote is formed by the fusion of two uni-nucleate cells, which must, however, from a phylogenetic view-point, be considered coenogametes. The production of the peculiar beak-cells is clearly a late specialization and makes these coenogametes of the second of Davis's types, namely, where only a portion of the nuclear material is functional.

If the evolutionary line thus indicated be the true one, it is reasonable to expect that Empusa, as the primitive form, will show a possible derivation from other Phycomycetes. The close agreement of the methods of zygospore-formation, both as to general morphology and as to cytological conditions, between Entomophthora and the Mucorales, which has been pointed out above, indicates relationship in that direction. If we consider such a form as Sporodinia, we find hyphae with very numerous small nuclei; a sporangium forming a number of uni-nucleate spores; and zygospores formed by the fusion of primitive coenogametes. Apogamy in this species is of frequent occurrence; and it is reasonable to assume that the azygospores thus formed are multi-nucleate. Comparing with this the conditions in Empusa, we see at once a possible line of derivation. The hyphal bodies, into which, on account of the peculiar endophytic habit, the hyphae break, contain many small nuclei, which are, however, less numerous and larger than those of Sporodinia. Thaxter ('88) has already shown that the "conidium" of Empusa might be regarded as a sporangium. It is clear therefore that, just as the coenogamete is a gametangium into which has been extended the coenocytic habit and which accordingly functions as a unit, so the "conidium" of Empusa is a sporangium into which has been extended the coenocytic habit and which likewise functions as a unit. Apogamy has become constant in Empusa, resulting in the formation of multi-nucleate azygospores. The close relationship of Empusa and Sporodinia seems undeniable.

We may now sum up the line of development indicated in this paper.

I. Sporodinia: Hyphae non-septate, with very numerous very small nuclei. Non-sexual reproduction by a sporangium producing numerous uni-nucleate spores. Resting-spore formed by the fusion of primitive coenogametes with very numerous nuclei. Apogamy frequent.

II. Empusa: Hyphae breaking into hyphal bodies with numerous small nuclei irregularly distributed. Non-sexual reproduction by a multi-nucleate "conidium" which is morphologically a coenocytic sporangium. Apogamy constant.

III. Entomophthora: Hyphae either breaking into hyphal bodies after passing through a septate condition or else permanently septate, with few large nuclei regularly spaced. Tendency toward nuclear reduction extended to the non-sexual reproduction resulting in a uni-nucleate coenosporangium. Resting-spores formed by the fusion of primitive coenogametes with few nuclei. Apogamy occasional.

IV. Basidiobolus: Hyphae septate, breaking into hyphal bodies only under abnormal conditions, with a single nucleus in each cell. Non-sexual reproduction as in Entomophthora. Resting-spores formed by the fusion of coenogametes of a specialized type, which through the extension of the tendency to nuclear-reduction have become uni-nucleate. Apogamy absent.

These three genera of Entomophthoraceae thus form a specialized line, derived from a Mucor-like ancestry, and reaching a high stage in the development of certain well-defined evolutionary tendencies.

Summary.

Material of the following species was studied during the present investigation: *Entomophthora Americana*, *E.* "*x*," *E. Geometralis*, *E. echinospora*, *E. rhizospora*, *E. Fresenii*, and *Empusa Grylli*.

The nucleus of Entomophthora shows a well-developed structure. There is a relatively small chromatin-nucleolus surrounded by a more or less dense zone of chromatin granules. The division of the nucleus is by a well-developed process of mitosis.

During mitosis, spherical chromosomes are organized by the direct aggregation of the chromatin-granules, no spireme stage being present. In *E. Americana*, eight chromosomes were counted with reasonable certainty.

The linin-fibres freed by this process of aggregation separate into two groups which migrate to the respective poles of the nucleus, forming a typical, intranuclear, bipolar spindle, without centrosomes.

The conidia of Empusa are multi-nucleate, and of Entomophthora, uni-nucleate. No nuclear-division is present in the process of conidium-formation.

The zygospores of Entomophthora are formed by the fusion of multinucleate hyphal bodies, which are coenogametes of a primitive type, since all of the nuclear material is retained in the zygote. No fusion of the nuclei takes place during the first three months after formation. Whether or not such fusion in pairs takes place at the time of germination it has been impossible to determine, owing to the difficulty of germinating the zygospores under artificial conditions.

The epispore of the zygote is derived directly from the walls of the hyphal bodies. The endospore is formed by the direct transformation of an outer zone of the cytoplasm of the zygote.

In *Empusa Grylli* the azygospores are formed by the budding out of a hyphal body. The entire nuclear and cytoplasmic contents of the hyphal body passes into the azygospore, which is therefore always multi-nucleate. No division and no fusion of these takes place.

The cytological evidence suggests the derivation of the Entomophthoraceae from a Mucor-like ancestry, with the extension of the coenocytic habit to the non-sexual methods of reproduction, and with the progressive reduction in the number of nuclei, with the increasing efficiency of the survivors, a process beginning in Empusa, carried further in Entomophthora, and reaching its highest expression in Basidiobolus.

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Bibliography.

Bail, Th.

'69. Ueber Pilzepizootien der forstverheerenden Raupen. Schriften d. naturforsch. Gesel. Danzig, Vol. 2, pt. 2, pp. 1–25, pl. 1.

Brefeld, O.

'84. Conidiobolus utriculosus und C. minor n. spp. Untersuchungen aus dem Gesammtgebiete Mykologie, Pt. 6, p. 35.

Cavara, F.

'99ᵃ. I nuclei delle Entomophthoreae in ordine alla filogenesi di queste piante. Bull. Soc. bot. italiana (1899), pp. 55–60.

'99ᵇ. Osservazioni citologiche sulle Entomophthoreae. Nuovo Giornale botanico italiano, Vol. 6, pp. 411–466, pl. 4, 5.

Chamberlain, C. J.

:01. Methods in Plant Histology. Chicago, Ill. 1901.

Cohn, F.

'75. Ueber eine neue Pilzkrankeit der Erdraupen. Beiträge z. Biol. d. Pflanzen, Vol. 1, Pt. 1, pp. 58–84, pl. 45.

Davis, B. M.

:00. The Fertilization of Albugo candida. Bot. Gazette, Vol. 29, pp. 297–311, pl. 22.

:03. Oogenesis in Saprolegnia. Bot. Gazette, Vol. 35, pp. 233 and 320, pl. 9, 10.

:04. Studies on the Plant Cell. Amer. Naturalist, Vols. 38, 39.

Eidam, E.

'87. Basidiobolus, eine neue Gattung der Entomophthoraceen. Beiträge z. Biol. d. Pflanzen, Vol. 4, pp. 181–251, pl. 9–12.

Fairchild, D. G.

'97. Ueber Kerntheilung und Befruchtung bei Basidiobolus. Jahr. wiss. Bot., Vol. 30, pp. 285–296, pl. 13, 14.

Gerassimow, J. J.

:01. Ueber den Einfluss des Kerns auf das Wachstum der Zelle. Bull. Soc. Imp. Nat., Vol. 15, pp. 185–220, pl. A, B.

:02. Die Abhängigkeit der Grösse der Zelle von der Menge der Kernmasse. Zeitschr. für allg. Physiol., Vol. 1, pp. 220–258.

:04. Ueber die Grösse der Zellkerns. Beih. zum Bot. Centr., Vol. 18, p. 43.

Gruber, E.

:01. Ueber das Verhalten der Zellkerne in den Zygospore von Sporodinia grandis. Berichte d. Deutsch Bot. Gesell., Vol. 19, pp. 51–55, pl. 2.

Harper, R. A.

:05. Sexual Reproduction and the Organization of the Nucleus in certain Mildews. Carnegie Inst., No. 37, pp. 1–87, pl. 1–7.

Hertwig, R.

:03. Ueber Korrelation von Zell und Kerngrösse, usw. Biol. Centr., Vol. 23, pp. 49 and 108.

Lawson, A. A.

:03. On the Relationship of the Nuclear Membrane to the Protoplast. Bot. Gazette, Vol. 35, pp. 305–317, pl. 15.

Lindau, G.

'97. Zur Entwickelung von Empusa Aulicae, Reich. Hedwigia, Vol. 36, pp. 291–296.

Lowenthal, W.

:03. Beiträge zur Kenntniss des Basidiobolus lacertae, Eidam. Archiv. f. Protistenkunde, Vol. 2, pp. 369–420, pl. 10, 11.

Maupas, E.

'79. Sur quelques protorganismes animaux et vegetaux multinuclées. Compte Rendus, Vol. 89, pp. 250–253.

Mitzkewitsch, L.

'98. Ueber die Kerntheilung bei Spirogyra. Flora, Vol. 85, pp. 81–85, pl. 5.

Montgomery, T. H.

'98. Spermatogenesis in Pentatoma up to the formation of the Spermatid. Zool. Jahrb. (Abth. Anat.), Vol. 12, pp. 1–82, pl. 1–5.

Raciborski, M.

'96[a]. Ueber den Einfluss äusserer Bedingungen auf die Wachstumsweise der Basidiobolus ranarum. Flora, Vol. 82, pp. 107–132.

'96[b]. Mykologische Studien: Karyokinese bei Basidiobolus; usw. Anzeiger der Akad. Wissenchaften in Krakaw (1896), pp. 377–386, pl. 1.

Riddle, L. W.

:06. Contributions to the Cytology of the Entomophthoraceae: Preliminary Communication. Rhodora, Vol. 8, pp. 67, 68.

Ruhland, W.

:03. Studien ueber die Befruchtung der Albugo Lepigoni und einiger Peronosporeen. Jahr. wiss. Bot., Vol. 39, pp. 135–166, pl. 2, 3.

Schroeter, J.

'86. Entomophthorei. In Krytogamen-Flora von Schlesien, Vol. 3, p. 217.

Stevens, F. L.

:01. Gametogenesis and Fertilization in Albugo. Bot. Gazette, Vol. 32, pp. 77, 157, and 286, pl. 1–4.

Stevens, F. L., and Stevens, A. C.

:03. Mitosis in the Primary Nucleus in Synchytrium decipiens. Bot. Gazette, Vol. 35, pp. 405–415, pl. 16, 17.

Thaxter, R.

'88. The Entomophthoreae of the United States. Memoirs Boston Soc. Nat. Hist., Vol. 4, pp. 133–199, pl. 14–21.

Vuillemin, P.

'87. Études biologiques sur les Champignons. Bull. Soc. Sci. Nancy (1887), pp. 1–126, pl. 1–6.

:00. Développement des Azygospores chez les Entomophthorées. Comptes Rendus, Vol. 29, pp. 670–684, pl. 6.

Wager, H.

'98. The Nucleus of the Yeast Plant. Annals of Bot., Vol. 12, pp. 499–537, pl. 29, 30.

Wolfe, J. J.

:04. Cytological Studies on Nemalion. Annals of Bot., Vol. 18, pp. 607–630, pl. 40, 41.

Woycicki, Z.

:04. Einige neue Beiträge zur Entwicklungs-geschichte von Basidobolus ranarum, Eidam. Flora, Vol. 93, pp. 87–97, pl. 4.

Chamberlain, C. J.

:01. Methods in Plant Histology. Chicago, Ill. 1901.

Cohn, F.

'75. Ueber eine neue Pilzkrankeit der Erdraupen. Beiträge z. Biol. d. Pflanzen, Vol. 1, Pt. 1, pp. 58–84, pl. 45.

Davis, B. M.

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:04. Studies on the Plant Cell. Amer. Naturalist, Vols. 38, 39.

Eidam, E.

'87. Basidiobolus, eine neue Gattung der Entomophthoraceen. Beiträge z. Biol. d. Pflanzen, Vol. 4, pp. 181–251, pl. 9–12.

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Gerassimow, J. J.

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:02. Die Abhängigkeit der Grösse der Zelle von der Menge der Kernmasse. Zeitschr. für allg. Physiol., Vol. 1, pp. 220–258.

:04. Ueber die Grösse der Zellkerns. Beih. zum Bot. Centr., Vol. 18, p. 43.

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:03. Ueber Korrelation von Zell und Kerngrösse, usw. Biol. Centr., Vol. 23, pp. 49 and 108.

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Lindau, G.

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Raciborski, M.

'96[a]. Ueber den Einfluss äusserer Bedingungen auf die Wachstumsweise der Basidiobolus ranarum. Flora, Vol. 82, pp. 107–132.

'96[b]. Mykologische Studien: Karyokinese bei Basidiobolus; usw. Anzeiger der Akad. Wissenchaften in Krakaw (1896), pp. 377–386, pl. 1.

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:06. Contributions to the Cytology of the Entomophthoraceae: Preliminary Communication. Rhodora, Vol. 8, pp. 67, 68.

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'86. Entomophthorei. In Krytogamen-Flora von Schlesien, Vol. 3, p. 217.

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:01. Gametogenesis and Fertilization in Albugo. Bot. Gazette, Vol. 32, pp. 77, 157, and 286, pl. 1–4.

Stevens, F. L., and Stevens, A. C.

:03. Mitosis in the Primary Nucleus in Synchytrium decipiens. Bot. Gazette, Vol. 35, pp. 405–415, pl. 16, 17.

Thaxter, R.

'88. The Entomophthoreae of the United States. Memoirs Boston Soc. Nat. Hist., Vol. 4, pp. 133–199, pl. 14–21.

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'87. Études biologiques sur les Champignons. Bull. Soc. Sci. Nancy (1887), pp. 1–126, pl. 1–6.

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Woycicki, Z.

:04. Einige neue Beiträge zur Entwicklungs-geschichte von Basidobolus ranarum, Eidam. Flora, Vol. 93, pp. 87–97, pl. 4.

EXPLANATION OF PLATES.

All the figures were drawn at stage-level with the aid of an Abbé camera, and with a Zeiss apochromatic 2 mm. objective and compensating ocular 4 (= × 500), 6 (= × 750), or 8 (= × 1000).

PLATE I.

Entomophthora Americana.

Figures 1–10. Mitosis (× 1000).

FIGURE 1. Resting-nucleus.

FIGURE 2. Prophase. Beginning of chromatin aggregation.

FIGURE 3. Prophase. Later stage of aggregation.

FIGURE 4. Prophase. Formation of the chromosomes, exposing the radiating linin-fibres.

FIGURE 5. Metaphase. Linin-fibres migrating to the poles.

FIGURE 6. Late prophase, showing eight chromosomes.

FIGURE 7. Early anaphase, showing typical spindle, with the daughter-chromosomes being drawn toward the poles.

FIGURE 8. Late anaphase. The daughter-chromosomes have reached the poles; the lower pole shows eight chromosomes.

FIGURE 9. Telophase. Reconstruction of the daughter-nuclei, which are separated by a vacuole.

FIGURE 10. Telophase. Separation of the daughter-nuclei by constriction.

Figures 11–14. Zygospore-formation (× 750).

FIGURE 11. Lateral fusion of the gametes, with budding of the spore from one of the gametes, the several nuclei of the hyphal bodies passing into the young zygospore (form "*x*").

FIGURE 12. Fusion of the gametes by their tips, with budding of the spore at the point of union.

FIGURE 13. Young zygospore before the formation of the cross-wall, with an attached empty hyphal body.

FIGURE 14. The same after the formation of the cross-wall. Figures 13 and 14 show the multi-nucleate condition.

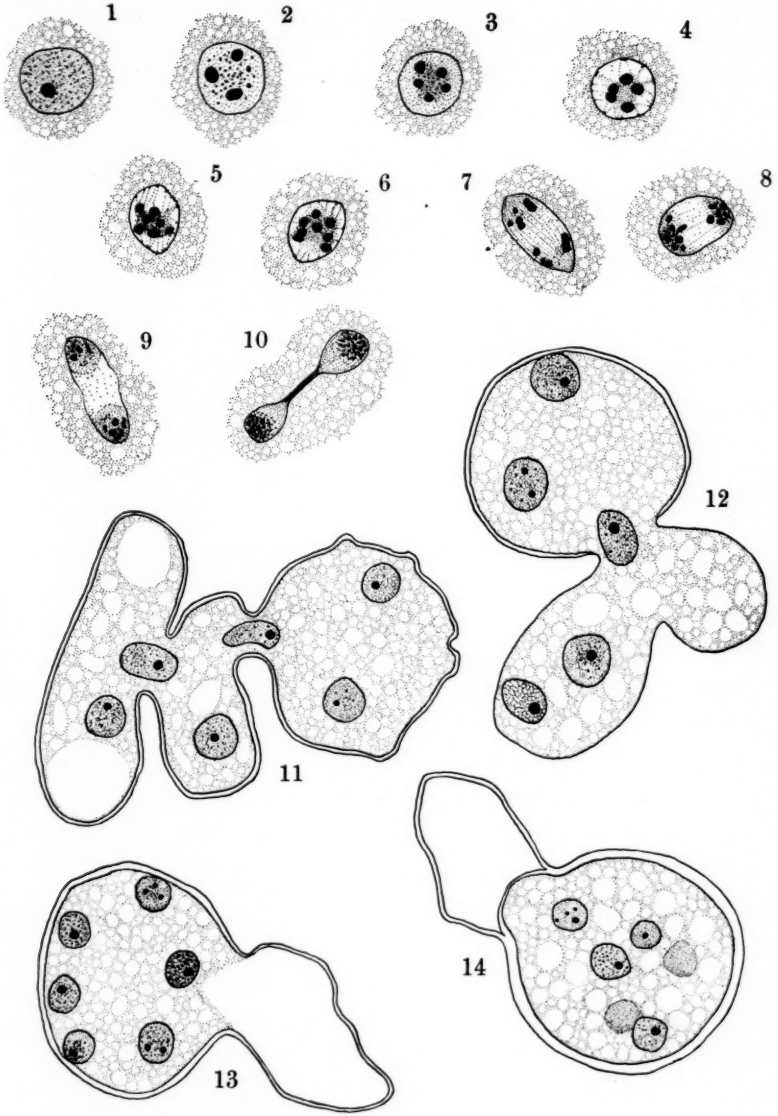
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PLATE II.

FIGURE 15. *Entomophthora "x."* Young zygospore, showing stage in the formation of the endospore (× 750).

FIGURE 16. *Entomophthora "x."* Mature zygospore, three months after formation; fixed in hot sublimate-acetic (× 750).

FIGURE 17. *E. Geometralis.* Septate conidiophore (× 500); fixed in Flemmings.

FIGURE 18. *E. Geometralis.* Mature conidium (× 1000).

FIGURE 19. *E. Geometralis.* Large, elongated nucleus in the conidiophore (× 1000).

Figures 20–22. *E. echinospora.* Zygospore-formation (× 750).

FIGURE 20. Young zygospore, showing method of formation.

FIGURE 21. Zygospore, after the formation of the cross-wall.

FIGURE 22. Mature zygospore, three months after formation.

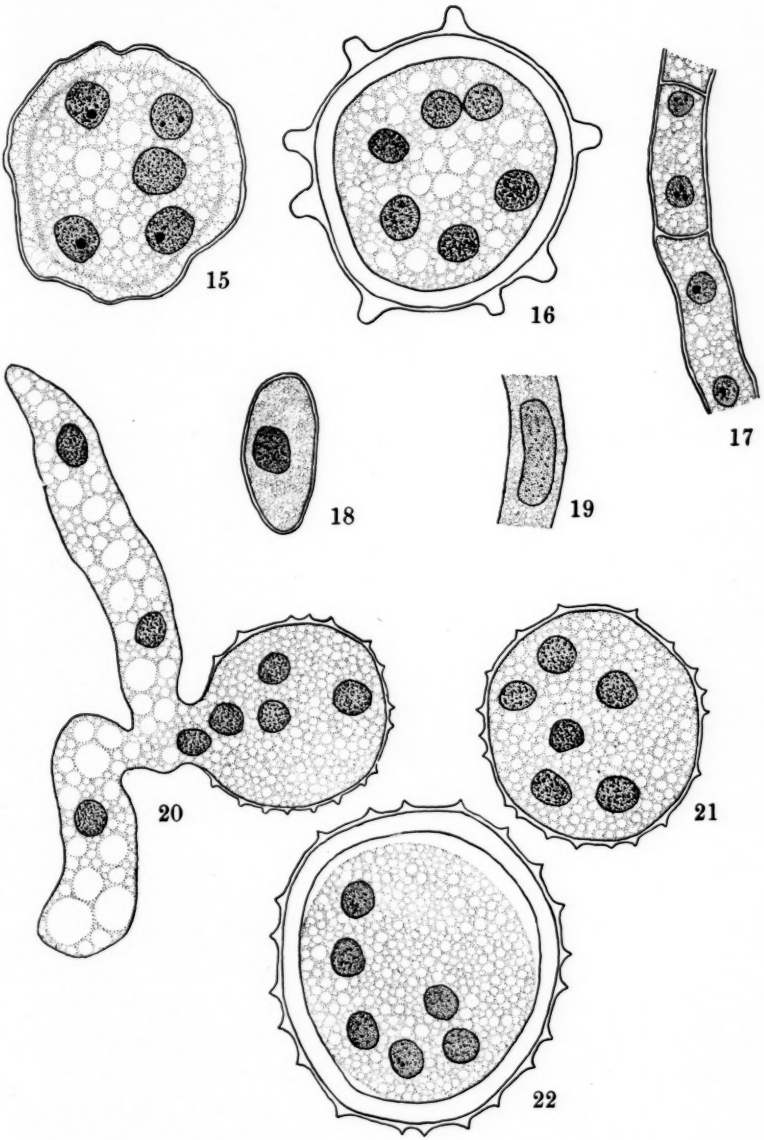
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PLATE III.

Empusa Grylli.

FIGURE 23. Young hyphal body (× 750).

FIGURE 24. Upper portion of conidiophore, showing the formation of the conidium (× 750).

FIGURE 25. Mature conidium (× 1000).

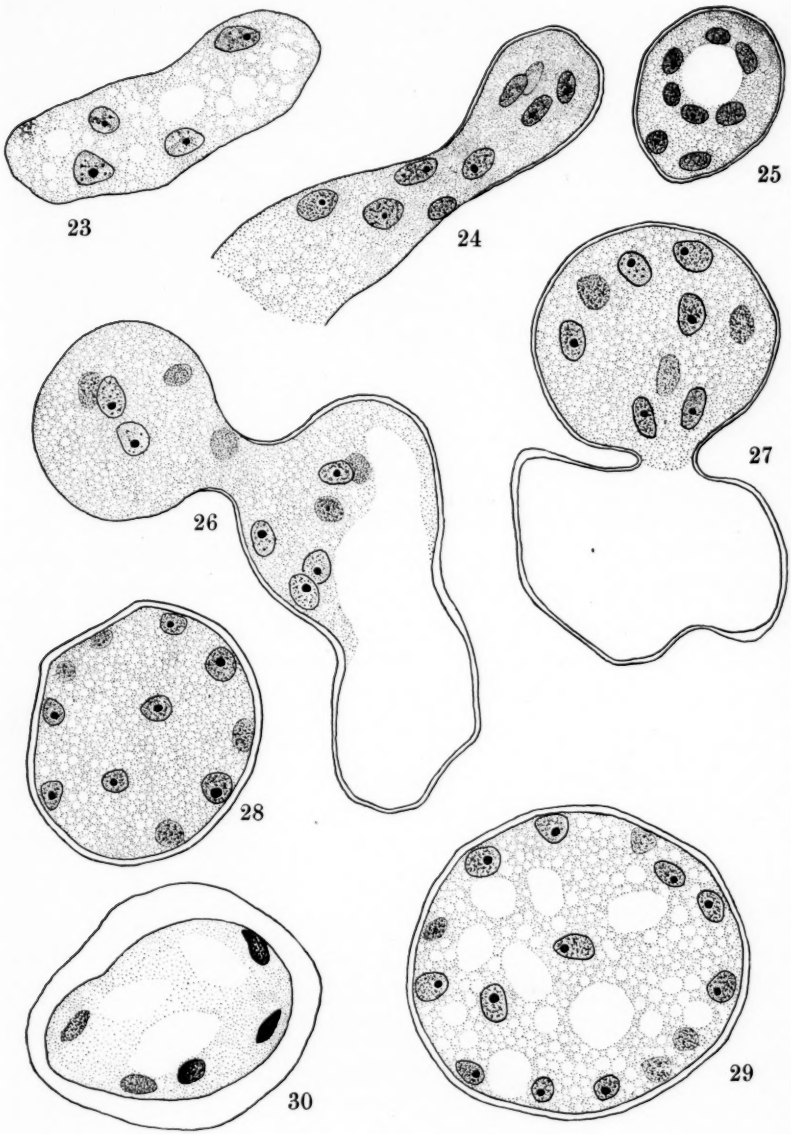
FIGURE 26. Young azygospore, showing a stage in the passage of the contents of the hyphal body into the young spore (× 750).

FIGURE 27. Young azygospore, before the formation of the cross-wall, showing the multi-nucleate condition and the attached empty hyphal body (× 750).

FIGURE 28. Mature azygospore, just after formation (× 750).

FIGURE 29. Mature azygospore, three months after formation, showing that no changes in the nuclei have taken place (× 750).

FIGURE 30. Encysted hyphal body; poor fixation due to the thick and resistant wall (× 750).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